NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF FUMONISIN B_1

(CAS NO. 116355-83-0)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

December 2001

NTP TR 496

NIH Publication No. 01-3955

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration (FDA); and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies on fumonisin B_1 were conducted at the NCTR under an interagency agreement between the FDA and the NIEHS. The studies were designed and monitored by a Toxicology Study Selection and Review Committee, composed of representatives from the NCTR and other FDA product centers, NIEHS, and other *ad hoc* members from other government agencies and academia. The studies were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with FDA Good Laboratory Practice Regulations.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

Details about ongoing and completed NTP studies are available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov. Abstracts of all NTP Technical Reports and full versions of the most recent reports and other publications are available from the NIEHS' Environmental Health Information Service (EHIS) http://ehis.niehs.nih.gov (800-315-3010 or 919-541-3841). In addition, printed copies of these reports are available from EHIS as supplies last. A listing of all NTP reports printed since 1982 appears on the inside back cover.

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CONTRIBUTORS

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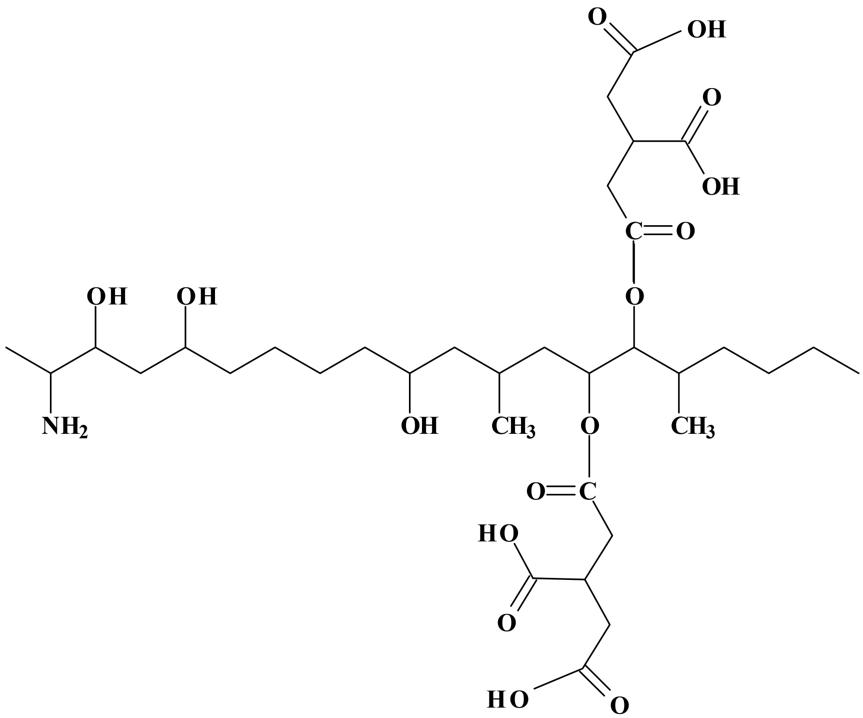
ABSTRACT



FUMONISIN B_1

CAS No. 116355-83-0

Chemical Formula: $C_{34}H_{59}NO_{15}$ Molecular Weight: 721.838

Synonyms: 1,2,3-Propanetricarboxylic acid, 1,1′-[1-(12-amino-4,9,11-trihydroxy-2-methyltridecyl)-2-(1-methylpentyl)-1,2-ethanediyl]ester; macrofusin

Fumonisin B_1 is a mycotoxin produced by the fungus *Fusarium moniliforme*, one of the major species found in corn. There are no known commercial or medical uses of fumonisin B_1. Fumonisin B_1 was nominated by the FDA Center for Food Safety and Applied Nutrition for study because of its occurrence in corn and corn-based products in the United States and its toxicity in field exposure of horses and pigs. Male and female F344/N Nctr BR rats and $B6C3F_1$/Nctr BR (C57BL/6N × C3H/HeN MTV^-) mice were exposed to fumonisin B_1 (92% pure) in feed for 28 days or (greater than 96% pure) for 2 years.

28-DAY STUDY IN RATS

Groups of 10 male and 10 female rats were fed diets containing 0, 99, 163, 234, or 484 ppm fumonisin B_1 for 28 days. There were no exposure-related deaths in rats. The mean body weights of the 484 ppm groups were significantly less (−16%) than those of the

controls. Dietary concentrations of 99, 163, 234, and 484 ppm fumonisin B_1 resulted in average daily doses of 12, 20, 28, and 56 mg fumonisin B_1/kg body weight for males and females.

Additional groups of male and female rats were exposed to the same concentrations of fumonisin B_1 for 28 days for clinical pathology studies. The concentrations of creatinine, cholesterol, triglycerides, and total bile acids, as well as activities of the enzymes alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, and γ-glutamyltransferase, were generally significantly greater in the 484 ppm groups than in the control groups at all time points, indicating hyperlipidemia and a hepatic effect. Fumonisin B_1 is an inhibitor of ceramide synthase, resulting in an interruption of *de novo* sphingolipid synthesis. This enzyme inhibition results in increased levels of sphinganine (or increased sphinganine:sphingosine ratio) in tissues and urine. Urinary sphinganine was increased in groups of males exposed to 163 ppm or greater, while urinary sphinganine was increased in all exposed groups of females.

The kidney weights, relative to body weight, of all exposed groups of rats were less than those of the control groups, decreasing by approximately 11% in the females and 20% in the males. Apoptosis and degeneration of the kidney were observed in all exposed males and in most females exposed to 163 ppm or greater. The incidences of minimal to mild apoptosis, degeneration, and mitotic alteration of the liver were significantly increased in 234 and 484 ppm males and in females exposed to 163 ppm or greater. The incidences of bile duct hyperplasia were significantly increased in males and females in the 484 ppm groups. In the core study, male rats in all exposed groups and females exposed to 163 ppm or greater had significantly increased percentages of hepatocytes in one or more proliferative (non-G_0) states.

28-Day Study in Mice

Groups of 12 male and 12 female mice were fed diets containing 0, 99, 163, 234, or 484 ppm fumonisin B_1 for 28 days. There were no exposure-related deaths in mice. The mean body weights of the 484 ppm groups of males were significantly less than those of the controls. Feed consumption by males exposed to 484 ppm was less than that by the controls; dietary concentrations of 99, 163, 234, and 484 ppm fumonisin B_1 resulted in average daily doses of approximately 19, 31, 44, and 93 mg/kg for males and 24, 41, 62, and 105 mg/kg for females.

Additional groups of male and female mice were exposed to the same concentrations of fumonisin B_1 for 28 days for clinical pathology studies. Cholesterol and total bile acid concentrations and alanine aminotransferase and alkaline phosphatase activities were increased at 484 ppm, indicating hyperlipidemia and a hepatic effect. Urinary sphinganine concentrations and sphinganine/sphingosine ratios were increased in 484 ppm male mice.

In 484 ppm males and all exposed groups of females, the incidences of hepatocellular necrosis, diffuse periportal hypertrophy, and diffuse centrilobular hyperplasia, as well as hyperplasia of the bile canaliculi and Kupffer cells, were generally significantly greater than those in the controls. Core study males exposed to 99, 163, or 234 ppm had significantly increased incidences of hepatocellular cytoplasmic alteration. Hepatocytes of 484 ppm male mice and all exposed groups of female mice were induced into proliferative (non-G_0) states.

2-Year Study in Rats

Groups of 48 male and 48 female rats (40 for 5 ppm groups) were fed diets containing 0, 5, 15, 50, or 150 ppm fumonisin B_1 (males) or 0, 5, 15, 50, or 100 ppm fumonisin B_1 (females) (equivalent to average daily doses of approximately 0.25, 0.76, 2.5, or 7.5 mg/kg to males and 0.31, 0.91, 3.0, or 6.1 mg/kg to females) for 105 weeks. Additional groups of four male and four female rats were exposed to the same concentrations as the core study animals and were evaluated at 6, 10, 14 or 26 weeks.

Survival, Body Weights, and Feed Consumption

Survival, mean body weights, and feed consumption of exposed male and female rats were generally similar to the controls throughout the study.

Clinical Pathology Findings

Sphinganine/sphingosine ratios were increased in the urine of 15, 50 and 150 ppm males and 50 and 100 ppm females exposed to fumonisin B_1 for up to 26 weeks. The sphinganine/sphingosine ratios were also increased in kidney tissue of 50 and 150 ppm males (85- and 119-fold) and 50 and 100 ppm females (7.8- and 22-fold) at 2 years.

Cell Proliferation Analyses

Renal tubule epithelial cell proliferation was increased in 50 and 150 ppm male rats exposed to fumonisin B_1 for up to 26 weeks. Renal tubule epithelial cell proliferation was marginally increased in 100 ppm females.

Organ Weights and Pathology Findings

Kidney weights of 50 and 150 ppm males were less than those of the controls at 6, 10, 14, and 26 weeks and at 2 years. Kidney weights of 100 ppm females were less than those of the controls at 26 weeks, and kidney weights of 15, 50, and 100 ppm females were less than those of the controls at 2 years.

At 2 years, there was a significant increase in the incidences of renal tubule adenoma from none in the groups receiving 15 ppm or less to five of 48 in 150 ppm males. Renal tubule carcinomas were not present in male rats receiving 15 ppm or less and occurred in seven of 48 and 10 of 48 male rats in the 50 and 150 ppm groups, respectively. Incidences of apoptosis of the renal tubule epithelium were generally significantly increased in males exposed to 15 ppm or greater for up to 26 weeks. The incidences of focal renal tubule epithelial hyperplasia were significantly increased in 50 and 150 ppm males at 2 years.

2-YEAR STUDY IN MICE

Groups of 48 male and 48 female mice were fed diets containing 0, 5, 15, 80, or 150 ppm (males) or 0, 5, 15, 50, or 80 ppm (females) fumonisin B_1 (equivalent to average daily doses of approximately 0.6, 1.7, 9.7, or 17.1 mg/kg to males or 0.7, 2.1, 7.1, or 12.4 mg/kg to females) for 105 weeks. Additional groups of four male and four female mice were exposed to the same concentrations as the core study animals and were evaluated at 3, 7, 9, or 24 weeks.

Survival, Body Weights, and Feed Consumption

Survival of males and females in the 15 ppm groups and of 5 ppm females was significantly greater and survival of 80 ppm males and females was significantly less than that of the control groups. Mean body weights and feed consumption of exposed mice were generally similar to the controls.

Organ Weights and Pathology Findings

Liver weights, relative to body weight, were increased 1.3- and 2.9-fold in 50 and 80 ppm females at 2 years. At 2 years, the incidences of hepatocellular adenoma in 50 and 80 ppm females were significantly greater than those in the controls and occurred with a positive trend. Similarly, the incidences of hepatocellular carcinoma increased from none in the groups receiving 0, 5, or 15 ppm fumonisin B_1 to 10 of 47 females at 50 ppm and nine of 45 females at 80 ppm. The incidences of hepatocellular hypertrophy were significantly increased in 15, 80, and 150 ppm males and in 50 and 80 ppm females at 2 years. The incidences of hepatocellular apoptosis were significantly increased in 50 and 80 ppm females at 2 years.

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *clear evidence of carcinogenic activity** of fumonisin B_1 in male F344/N rats based on the increased incidences of renal tubule neoplasms. There was *no evidence of carcinogenic activity* of fumonisin B_1 in female F344/N rats exposed to 5, 15, 50, or 100 ppm. There was *no evidence of carcinogenic activity* of fumonisin B_1 in male B6C3F$_1$ mice exposed to 5, 15, 80, or 150 ppm. There was *clear evidence of carcinogenic activity* of fumonisin B_1 in female B6C3F$_1$ mice based on the increased incidences of hepatocellular neoplasms.

The sphinganine/sphingosine ratios were increased in the urine and the kidney tissue of rats receiving diets containing fumonisin B_1. There was evidence of apoptosis and increased cell proliferation of the renal tubule epithelium in exposed rats, particularly in those groups of males that developed renal tubule neoplasms. Increased incidences of hyperplasia of the renal tubule epithelium also occurred in these groups of male rats.

In mice exposed to the higher concentrations of fumonisin B_1, males and females had increased incidences of hepatocellular hypertrophy and females had increased incidences of hepatocellular apoptosis.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 12.

Summary of the 2-Year Carcinogenesis Studies of Fumonisin B_1

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Concentrations in feed	0, 5, 15, 50, or 150 ppm	0, 5, 15, 50, or 100 ppm	0, 5, 15, 80, or 150 ppm	0, 5, 15, 50, or 80 ppm
Body weights	Exposed groups similar to control group	Exposed groups similar to control group	Exposed groups similar to control group	Exposed groups similar to control group
Survival rates	16/48, 17/40, 25/48, 18/48, 25/48	25/48, 22/40, 24/48, 30/48, 29/48	41/48, 39/48, 45/48, 37/48, 42/48	35/48, 44/48, 46/48, 39/48, 28/48
Nonneoplastic effects	Kidney: renal tubule epithelial hyperplasia, focal (2/48, 1/40, 4/48, 14/48, 8/48)	None	Liver: hepatocellular hypertrophy (10/47, 9/47, 24/48, 25/48, 30/48)	Liver: hepatocellular hypertrophy (0/47, 0/48, 0/48, 27/47, 31/45); hepatocellular apoptosis (0/47, 0/48, 0/48, 7/47, 14/45)
Neoplastic effects	Kidney: renal tubule adenoma (0/48, 0/40, 0/48, 2/48, 5/48); renal tubule carcinoma (0/48, 0/40, 0/48, 7/48, 10/48); renal tubule adenoma or carcinoma (0/48, 0/40, 0/48, 9/48, 15/48)	None	None	Liver: hepatocellular adenoma (5/47, 3/48, 1/48, 16/47, 31/45); hepatocellular carcinoma (0/47, 0/48, 0/48, 10/47, 9/45); hepatocellular adenoma or carcinoma (5/47, 3/48, 1/48, 19/47, 39/45)
Level of evidence of carcinogenic activity	Clear evidence	No evidence	No evidence	Clear evidence

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on fumonisin B_1 on 21 May 1999 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 21 May 1999, the draft Technical Report on the toxicology and carcinogenesis studies of fumonisin B_1 received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.R. Bucher reported that the 2-year studies on fumonisin B_1 were the first to come to the Subcommittee for peer review under an Interagency Agreement signed in 1992 between the Food and Drug Administration (FDA) and the National Institute of Environmental Health Sciences (NIEHS) to support the performance of studies evaluating the toxicology and carcinogenic activity of chemicals and agents that were primarily of interest to the FDA.

Dr. P.C. Howard, NCTR, introduced the toxicology and carcinogenesis studies of fumonisin B_1 by noting that fumonisin B_1 is the most prevalent of a number of fungal metabolites of the *Fusaria* species found primarily on corn in the United States and around the world and reporting on the principal mode of action of fumonisin B_1 in interrupting sphingolipid synthesis. Dr. Howard then described the experimental design for 28-day and 2-year studies in rats and mice, including clinical chemistry indicators of hepatotoxicity, renal toxicity, sphingolipid metabolism, and measures of apoptotic activity. He reported on survival and organ and body weight effects and commented on compound-related neoplastic lesions in male rats and female mice and nonneoplastic lesions in male rats and male and female mice. The proposed conclusions for the 2-year studies were *clear evidence of carcinogenic activity* in male F344/N rats, *no evidence of carcinogenic activity* in female F344/N rats, *no evidence of carcinogenic activity* in male B6C3F$_1$ mice, and *clear evidence of carcinogenic activity* in female B6C3F$_1$ mice.

Dr. Fischer, a principal reviewer, agreed with the proposed conclusions. She asked for better definition of the core study group and the additional animals including which animals were being used for which part of the studies. Dr. Howard explained that the core animals were fed continuously for 28 days while the other animals were fasted overnight at intervals for collection of urine or blood, and this distinction would be clarified. Dr. Fischer thought the Abstract would be more complete if information were added indicating that fumonisin B_1 is an inhibitor of ceramide synthase and that this is responsible for the biological consequences of fumonisin B_1 exposure. Dr. Howard agreed. Dr. Fischer noted that because there are major species differences in response to fumonisin B_1 with regard to the particular target tissue affected there should be more discussion about the relevance of all of these animal studies to humans. Dr. Howard commented that the mechanisms of action of these different target organ effects are not well understood, and further, there is no validated biomarker for human exposure, so the relevance of animal to human findings would be hard to discern at this point.

Dr. Bailer, the second principal reviewer, agreed with the proposed conclusions although he thought the increases of the incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in female rats should be listed as an uncertain finding in the Abstract. Dr. Howard said that the 2% incidence in the high exposure group was not considered a significant enough increase, although this could be argued by some as fitting "equivocal evidence." Dr. Bailer questioned not doing complete histopathology on some of the mid exposure groups, clearly decreasing the sensitivity of detecting tumor onset and trend with a loss of dose-response information. Dr. Howard responded that this was the protocol agreed on for the study. However, knowing that liver and kidney were likely target organs, these organs were examined from intermediate exposure groups as well, and of course, animals dying before terminal sacrifice had complete histopathology, regardless of exposure group. Dr. Bucher commented that where there is good interaction among pathologists, study directors, and the test laboratory, there is always the ability to go back and cut in tissues as needed. Dr. Carlson commented that in view of toxicity to heart, lung, and esophagus in other species, one could argue that there should have been complete histopathology on these organs. Dr. Bailer said that incomplete histopathology may have led to some bizarre neoplasm patterns, e.g., the tumor burden for harderian gland adenomas or carcinomas in male mice. Dr. R.L. Kodell, NCTR, said that his opinion was that intermediate dose group data from animals dying before terminal sacrifice should not be used in a statistical analysis.

Dr. Davis, the third principal reviewer, agreed with the proposed conclusions. He had some concerns about the exposure concentration selection noting the lack of tumor response in female rats and male mice and the statement that female rats could have tolerated a higher exposure concentration. Dr. Howard responded that exposure concentrations for the 2-year study were selected based on multiple issues including the hepatotoxicity, nephrotoxicity, and literature information on mechanisms of target organ toxicity. Further, Dr. Davis criticized not doing 90-day studies in conjunction with the 2-year bioassay while relying on 90-day studies done by Voss *et al.* (1993). Dr. Howard stated that it was discussed whether or not to conduct another 90-day study, but since the study by Voss *et al.* (1993) was conducted with test material provided by the NTP and was under NTP guidance, the study was considered adequate for moving ahead to a 2-year study. Dr. Davis also expressed concern about the underfeeding by 30% of the mice in the 2-year study. Dr. Howard explained that the doses in the feed were accurate, but the mice consumed 30% less feed. As a partial explanation, it was realized about nine months into the study that the mice weighed less than the average NTP mouse at that point. The decision was made to continue the study. Dr. Howard said that a factor in the decision not to stop and restart was the cost of the material, $40,000 a gram, and the lengthy time (years) required to purify it.

In other discussion, Dr. Russo asked what human doses from contaminated corn would be in relation to 100 ppm doses in animals. Dr. Howard reported that in South Africa where esophageal cancer is seen and where corn is an everyday staple in the diet, estimates of human intake are around 0.2 mg/kg per day which is within a couple of orders of magnitude of the animal dose. Dr. Russo asked whether there were any pathologic changes in the esophagi of study animals. Dr. Howard responded that there were not, which is at variance with other reported rat studies in which hyperplasia was reported. Dr. Hecht stated that at least 50 nitrosamines can induce esophageal tumors in rats, which suggests that fumonisin B_1 may not be the agent responsible for esophageal cancer in humans. Dr. Howard acknowledged the possible presence of nitrosamines in fungally contaminated corn. He concluded that a proper epidemiological study has not yet been done with fumonisin; rather the best studies available are correlative.

Dr. Fischer moved that under the conditions of this study the Technical Report on fumonisin B_1 be accepted with revisions discussed and the conclusions as written for male rats and female mice, *clear evidence of carcinogenic activity*, and for females rats and male mice, *no evidence of carcinogenic activity*. Dr. Davis seconded the motion, which was accepted unanimously with eight yes votes. Drs. Bailer and Bus were not present for the vote.